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Biotest system for rapid evaluation of concrete resistance to sulfur-oxidizing bacteria*

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Concrete exposed to sewage or industrial waste in the presence of air and inorganic reduced sulfur compounds often degrades rapidly. Sulfur-oxidizing bacteria of the genus Thiobacillus *that generate sulfuric acid as the end product of their metabolism play an important role in this process. To evaluate the resistance of concrete to the activity of these microorganisms, a specially designed hydrogen sulfide chamber containing concrete test blocks was built. Temperature, humidity, hydrogen sulfide concentration, and exposure to aerosols of different thiobacilli are controlled in this chamber. Experiments show that the rate of concrete degradation is accelerated, and corrosion requiring at least 5 y in sewer systems was reproducibly demonstrated within nine months. With this system, the degradation rates corresponding to weight loss between 1 and 10% correlated most closely with densities between 10^6 and 10^8 cells/cm^2 of the bacterium* T. thiooxidans *on the surface of the concrete test specimens. Specific polar lipid components in the membranes of the thiobacilli can be used to monitor the number of these organisms on the surfaces of corroding concrete.*

Introduction

THE USE OF THE VERSATILE AND RELATIVELY INEXPENSIVE building material concrete in certain environments can lead to severe corrosion problems. In the presence of reduced forms of sulfur, oxygen, nitrogen, and a carbon source that can be carbon dioxide, reduced sulfur-oxidizing microorganisms can generate sulfuric acid. In the presence of sulfuric acid, gypsum can be created from calcium hydroxides and carbonates, often with disastrous weakening of the structure. This problem has been noted primarily in concrete sewer lines, particularly in warm and moderate climates.[1-4] In 1945, Parker described a biologically mediated corrosion mechanism for the Melbourne, Australia sewer system.[5] Microbially generated hydrogen sulfide is transported to the walls of sewer pipes and converted to sulfur, which is oxidized to sulfuric acid by the thiobacilli metabolism. That theory has been confirmed by this work.[4,6-12] While studying sulfuric acid attack on the sewer network of Hamburg (FRG), an *in situ* examination of the thiobacilli involved in the biocorrosion process revealed them to be *Thiobacillus intermedius*, *T. novellus*, *T. neopolitanus*, and *T. thiooxidans*.[6]

To study the corrosion process under controlled conditions, a chamber was constructed in which the temperature, humidity, hydrogen sulfide concentration, and exposure to aerosols of specific mixtures of these thiobacilli were accurately controlled. The corrosion was reproducibly accelerated in this chamber so that a process requiring over five years in the field could be observed in nine months. The test system thus became an excellent vehicle for monitoring both the resistance of different types of concrete as well as the effects of cell numbers and thiobacilli species on corrosion rates.[13] Preliminary evidence is also presented showing that the long process of viable counting of cultured thiobacilli isolated from the concrete surfaces can be supplemented or replaced by quantitative measurements of the *signature* components of polar lipids of these organisms.

Experimental procedure

Controlled exposure chamber

A stainless steel (SS) chamber was constructed in which 32 concrete specimens (60 × 11 × 7 cm in size and scored into 1.8-cm squares) were placed on end with the base standing in 10 cm of water. The water temperature was maintained at 30 C, and the pH was held at 7.0 by means of an automatic titration unit. The air space above the water was maintained at 30 C and 100% humidity, with 10 ± 1 ppm hydrogen sulfide as monitored by a gas chromatograph.[4] The blocks were periodically sprayed with thiobacilli cultures that had been isolated from the sewer system. The pH at the concrete surface was monitored by firmly attached pH strips on the concrete specimens. The strips were replaced every two weeks. Figure 1 shows a diagram of the test apparatus. Figure 2 shows the concrete test specimens and chamber.

Monitoring the corrosion

After a 3-month inoculation period, each specimen was sampled every 90 days for 3 to 4 periods. The surface cubes were broken off of the concrete test samples, transferred to sterile bottles containing 50 mL of sterile washing solution, and incubated in flasks rotated at 200 rpm on a shaker for 30 min. The resulting suspensions were used for dilution series, from which selective media for thiobacilli, bacterial heterotrophs, and fungi were inoculated. The cultural evaluation lasted four weeks. The weight loss of the cubes was monitored

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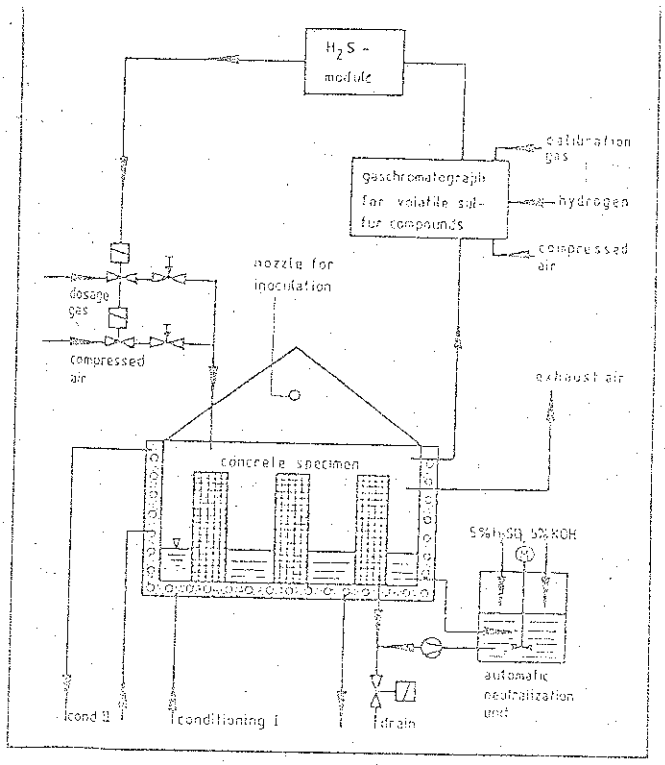

FIGURE 1 — Diagram of the constant temperature, humidity, and hydrogen sulfide *Biotest* chamber.

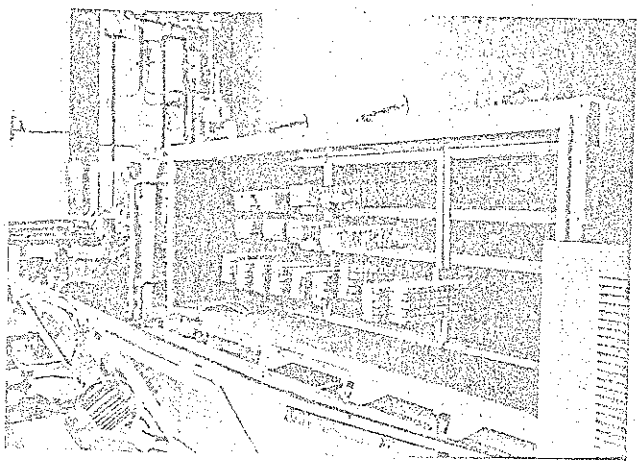

FIGURE 2 — Photograph of the *Biotest* chamber showing the concrete samples in place with the surface pH test strips.

at the beginning and end of the experiments. After shaking the cubes for 2 h in sterile washing solution, the cubes and dried washing solution were weighed. The weight loss equaled the weight of the dried corrosion products found in the washing solution divided into the sum of the dried corrosion products and dried cubes.

Monitoring bacterial growth

The most probable number technique was used to establish the microbes involved with the corrosion. For *T. intermedius* and *T. novellus*, the medium contained 5.0 g/L $Na_2S_2O_3$, 0.13 g/L $CaCl\cdot 2H_2O$, 1.0 g/L NH_4Cl, 1.02 g/L $MgSO_4\cdot 7H_2O$, 0.4 g/L KH_2PO_4, 0.6 g/L K_2HPO_4, 50 g/L trace metal solution [5 mL/L of ethylenediaminetetraacetic acid (EDTA), 2.2 g/L $ZnSO_4\cdot 7H_2O$, 5.5 g/L $CaCl_2$, 5.06 g/L $MnCl_2\cdot 4H_2O$, 5.0 g/L $FeSO_4\cdot 6H_2O$, 1.1 g/L $(NH_4)_6Mo_7O_{24}$, 1.57 g/L $CuSO_4\cdot 5H_2O$, and 1.61 g/L $CoCl_2\cdot 6H_2O$ in 1.0 L distilled water], 2 mg/L ferric-EDTA, and 24.4 mg/L biotin in 1.0 L distilled water with a final pH of 6.5. For *T. neopolitanus*, the medium contained 10 g/L $Na_2S_2O_3\cdot 5H_2O$, 0.8 g/L $MgSO_4\cdot 7H_2O$, 0.4 g/L NH_4Cl, 10 mL/L trace metal solution, 4.0 g/L KH_2PO_4, and 4.0 g/L K_2HPO_4 in 1.0 L distilled water. For *T. thiooxidans*, the medium contained 10.0 g/L $Na_2S_2O_3\cdot 5H_2O$, 2.0 g/L KH_2PO_4, 1.0 g/L CaCl, 0.2 g/L $MgSO_4\cdot 7H_2O$, 0.1 g/L $(NH_4)_2SO_4$, 0.04 g/L $CaCl_2\cdot 2H_2O$, 0.02 g/L $FeCl_3\cdot 6H_2O$, and 0.015 g/L $MnSO_4\cdot H_2O$ in 1.0 L distilled water. The washing solution was the *T. neapolitanus* medium without thiosulfate. The media were sterilized for 30 min at 110 C.

The cell counts were performed on serial dilutions in steps of 1 through 10. Five culture tubes with 2.5 mL of each medium were inoculated with 0.5 mL of the serial dilution steps and incubated on a rotary incubator at 30 C for aeration. The tests were evaluated after 3 weeks of incubation. Tests for *T. neapolitanus* and *T. thiooxidans* were considered positive if the pH values of the medium were below 4.0 and 2.0, respectively. To differentiate between *T. intermedius/novellus* and *T. neapolitanus*, which both grow on the *T. intermedius* medium, each test tube with turbidity and a pH below 4.0 was streaked on *T. intermedius/novellus* agar (salts plus 1.5% agar) and incubated at 30 C for 1 week. The test was considered positive for *T. intermedius/novellus* if the colonies were transparent and yellowish and negative if the colonies were opaque and white (*T. neapolitanus*).

Heterotrophic aerobic bacteria were scored after growth on agar plates containing DEV-gelatin agar (Merck,[1] FRG). The fungi were counted after growth on Sabouraud-Maltose agar (Merck, FRG).

Analysis for signature lipids

Specimens of corroded concrete and monocultures of the thiobacilli were extracted with a one-phase chloroform-methanol solvent, and the lipids were fractionated on silicic acid columns.[14,15] The polar lipid fraction recovered in methanol was subjected to mild alkaline methanolysis, and the products were partitioned against water. The water portion was analyzed for phosphate (total extractable phospholipid).[14] The lipid portion was fractionated into the acyl fatty acid methyl esters and hydroxy fatty acid methyl esters by thin layer chromatography; the esters were recovered and analyzed by capillary gas liquid chromatography and with structural confirmation by mass spectrometry.[16]

Results and discussion

Corrosion and surface pH of concrete

Three types of concrete exhibit different responses to the corrosive activities of the thiobacilli (Figure 3). All specimens had initial pH values between 9 and 11. With the resistant Portland cement shown in the upper panel of Figure 3, the pH decreased within 120 days to between 2.0 ± 0.3 and remained constant until the end of the experiment. The middle panel of Figure 3 shows Portland cement of intermediate resistance. The initial pH remained constant for 70 days, decreased to pH values between 2 and 3 in the next 45 days, and then remained at that pH until the end of the experiment. The lower panel shows the response of blast furnace-type cement with the poorest resistance to the microbial corrosion. The pH decreased to 3 within 50 days and continued to fall to 1.0 pH.

Cell counts of thiobacilli and corrosion of concrete

The cell counts of the three major groups of thiobacilli exhibit greatest levels in the most rapidly degrading concrete (lower panel of Figure 3). Although the lowest levels of *T. intermedius/novellus* appear to be associated with the most resistant concrete (upper panel of Figure 3), the best correlations between the degradation rates of the concrete and thiobacilli

[1]Registered trade name.

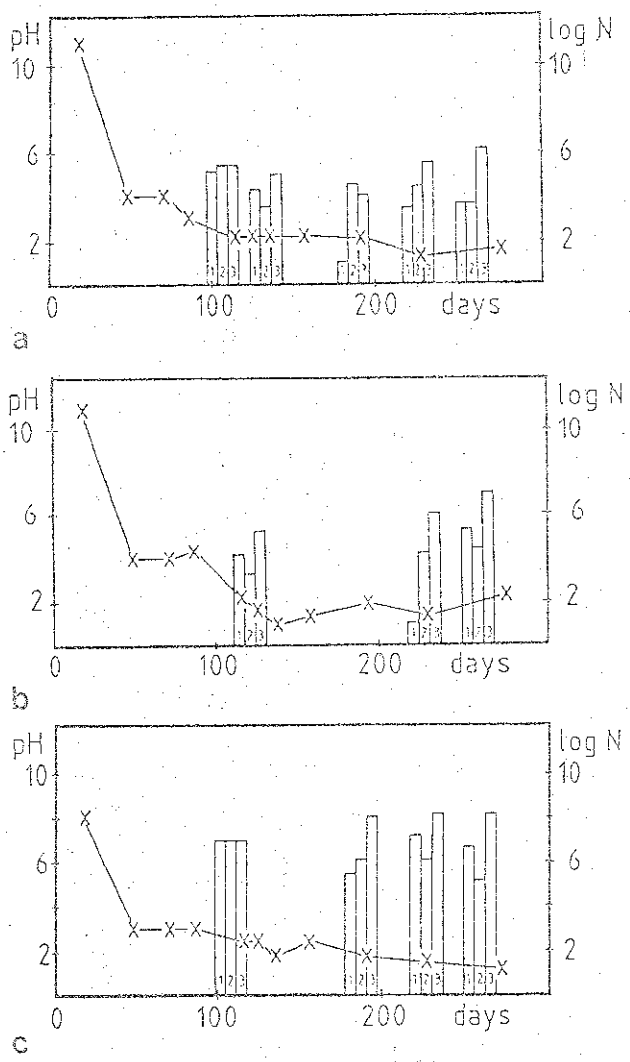

FIGURE 3 — Profile of the pH (X) and logarithms of the cell numbers of (a) *T. intermedius/novellus*, (b) *T. neapolitanus*, and (c) *T. thiooxidans* measured after 270 days in the test chamber (upper panel—resistant Portland cement; middle panel—intermediate resistant Portland cement; and lower panel—blast furnace cement of poorest resistance).

TABLE 1 — Relationship between biodegradation of concrete and number of *T. thiooxidans*

Corrosion grade	Weight loss % of total	Number of *T. thiooxidans* logarithm cells/cm²	Number analyzed
Negligible	0.7 ± 0.5	6.8 ± 0.6	3
Medium	2.3 ± 1.7	7.1 ± 0.7	6
Great	5.8 ± 2.9	7.7 ± 0.5	5

are with the most powerful acid-generating species, *T. thiooxidans* (see Table 1).

The plate counts of heterotrophic aerobic bacteria and fungi were independent of the concrete specimen tested.

Potential of signature lipid analysis of thiobacilli

Assay of the thiobacilli from the dilution tubes and culture plates requires at least four weeks and cannot distinguish between *T. intermedius* and *T. novellus*. Recently, the use of chemical assays of the lipid components of microbial consortia has been shown to provide a quantitative measure of the biomass and community structure without the necessity of isolating the organisms from the growth substrate or culture of the organisms once they are isolated.[17] The early work of Shively suggested that the thiobacilli lipids were sufficiently unusual to serve as signatures of these organisms.[18,19] Our work has shown that the thiobacilli polar lipids contain ester-linked and amide-linked fatty acids with unusual structures, such as monoenoic 15, 16, and 17 carbon fatty acids with unsaturations at 5 through 9 carbons from the methyl end of the molecules.[20] Most organisms have the unsaturation at a single position, usually at the 7 or 9 position from the methyl end. These organisms also contain unusually large proportions of cyclopropane fatty acids, with the three-membered ring between 7 and 8 in the 17 carbon and between 8 and 9 in the 19 carbon atom fatty acids. These lipids also contain unusual 2 hydroxyl monounsaturated, 10 and 11 methyl branched, 2 hydroxyl cyclopropane, 10 through 13 methoxy saturated, and 10 and 13 hydroxy saturated fatty acids. These fatty acids occur in proportions that allow the individual species to be differentiated from each other.[20] The unusual polar lipid fatty acids and the bound fatty acids from the lipopolysaccharide enable the acid-producing thiobacilli to be identified in samples from the test specimens and corroded concrete sewer samples from the field.[21]

Conclusions

It has proven possible to correlate in sewer pipes, in the field, and on concrete specimens in a strictly controlled hydrogen sulfide test chamber that the degree of concrete degradation directly correlates with the numbers of *T. thiooxidans*.[11,12] These organisms depress the pH of the concrete surface to values between 1 and 3 by their excretion of sulfuric acid. The development of a biochemical assay for the thiobacilli allows greater insight into the relationship between the metabolic activity of the organisms and the degradation of concrete. The development of this test chamber, which provides reproducible exposures to the biodegradative activity of the thiobacilli in reasonable timespans, has led to a biotest facility for this most versatile building material.

Acknowledgments

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